

Adaptations to the Deep-Sea Oxygen Minimum Layer: Oxygen Binding by the Hemocyanin of the Bathypelagic Mysid, *Gnathophausia ingens* Dohrn

N. K. SANDERS AND J. J. CHILDRESS

*Oceanic Biology Group, Marine Science Institute and Department of Biological Sciences,
University of California, Santa Barbara, California 93106*

Abstract. The bathypelagic mysid, *Gnathophausia ingens* Dohrn, lives aerobically at oxygen partial pressures as low as 6 torr in the oxygen minimum layer off southern California. This study is concerned with the O₂ binding properties of this mysid's hemocyanin and the function of the pigment in O₂ uptake at low P_{O₂}. The effect of temperature on *in vivo* hemolymph pH ($\Delta\text{pH}/\Delta T = -0.018$) was measured from 2.5 to 12.5°C. Hemocyanin concentration was estimated to be 24 mg/l, corresponding to an O₂ binding capacity of about 0.3 mmol O₂/l. Freezing of hemolymph samples significantly decreased the affinity and cooperativity of HcO₂ binding, necessitating the use of fresh hemolymph. The HcO₂ affinity was high (P₅₀ of 1.4 torr at 5.5°C, pH 7.87), allowing the loading of O₂ even at 6 torr. The cooperativity of HcO₂ binding was also high (n₅₀ = 3.5 at 5.5°C, pH 7.87); presumably allowing the pigment to function effectively as an O₂ transporter within the small P_{O₂} difference between the environment and the tissues. Temperature differences within the environmental range (2–10°C) had no significant effect on the oxygen affinity ($\Delta H = -6.7$ kJ/mol, pH 7.7) or on the cooperativity of O₂ binding. A large Bohr shift ($\Delta \log P_{50}/\Delta \text{pH} = -0.80$ to -0.81) was present at all temperatures. L-lactate produced moderate increases in HcO₂ affinity ($\Delta \log P_{50}/\Delta \log [\text{lactate}] = -0.13$ at pH 7.9) and in cooperativity. Regional and ontogenetic comparisons suggest that regional and ontogenetic differences in HcO₂ affinity occur in this species. This mysid has a hemocyanin of unusually high O₂ affinity and cooperativity of O₂ binding for a crustacean living at low temperatures, and this appears to be an adaptation for oxygen loading and transport at the cold,

low oxygen conditions in deep-sea oxygen minimum layers. The reduced temperature sensitivity of HcO₂ affinity may also be an adaptation to low oxygen.

Introduction

Zones of minimum oxygen are found at intermediate depths in most of the world's oceans and, although the oxygen partial pressure in some of these "oxygen minimum layers" is only a few torr, populations of pelagic metazoans exist there (Schmidt, 1925; Sewell and Fage, 1948; Banse, 1964). These oxygen minimum layers are pelagic habitats with stable conditions of continuously low oxygen and low temperature at intermediate depths (400–1000 m depth) over vast areas. Previous studies have shown that most of the pelagic crustaceans living off California, where P_{O₂} at the oxygen minimum is 6 torr, are able to do so aerobically by being unusually effective at extracting O₂ from water (Childress, 1968, 1971, 1975). This remarkable ability has been intensively studied in the lophogastrid mysid *Gnathophausia ingens* Dohrn (Childress, 1968, 1971; Belman and Childress, 1976).

Gnathophausia ingens is the largest entirely pelagic crustacean, and has a circumglobal distribution between 30°N and 30°S latitudes. The mature female (instar 13, estimated duration of 530 days) produces and carries a single brood at depths greater than 800 m (Childress and Price, 1978, 1983). The first two free-living instars (3 and 4, estimated durations of 95 days each) live at depths as shallow as 150–200 m. However, for much of its life (instars 5 to 10, "intermediate instars," estimated durations from 168 to 207 days each), *G. ingens* occurs at depths of about 400–800 m, corresponding to the depth range

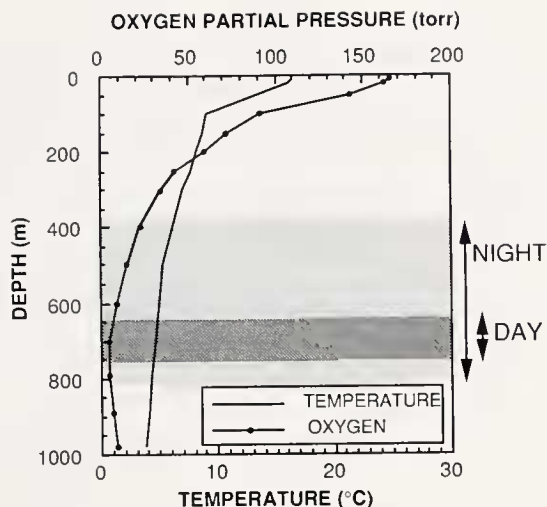


Figure 1. The day and night depth distribution patterns of *Gnathophausia ingens* shown with environmental temperatures and O₂ partial pressures off southern California.

of the oxygen minimum layer off southern California (Fig. 1, 6–20 torr O₂, 4–7°C) (Childress and Price, 1978, 1983). This species has limited anaerobic capacity, and is able to live aerobically at the lowest P_{O₂} it encounters off southern California (Childress, 1968; 1971), although it may use anaerobic metabolism briefly to support high activity levels at the lowest O₂ levels. Its ability to regulate its oxygen consumption to P_{O₂} values as low as 3 torr is due to its ability to maintain a high ventilatory flow (up to 8 body volumes min⁻¹), and simultaneously to remove a large fraction (50–80%) of the oxygen in the inhaled water (Childress, 1971). These abilities are made possible by the highly developed gills and circulatory system (Belman and Childress, 1976). Belman and Childress (1976) also showed that a high affinity, high cooperativity respiratory protein must be present to provide sufficient hemolymph oxygen carrying capacity and unloading at the very low P_{O₂} values at which these mysids live, although at the time of their studies no respiratory protein had been found in the order Mysidacea.

A preliminary report demonstrated the presence, in *Gnathophausia ingens*, of a hemocyanin having a high affinity for oxygen at 20°C (Freel, 1978), but the properties of this hemocyanin were not measured at environmentally appropriate temperatures or pH levels. The report by Freel is the only publication on a hemocyanin in the entire order Mysidacea. The high affinity at high temperature reported by Freel appears anomalous; *i.e.*, the O₂ affinity of hemocyanin normally increases greatly at low temperatures, so how could the hemocyanin be functional at the much lower environmental temperature? In fact, shallow-dwelling crustaceans living at lower temperatures have hemocyanins with lower O₂ affinities

as well as lower cooperativities, presumably to maintain a sufficient unloading of O₂ to their tissues (Redmond, 1968; Mangum, 1982; Mauro and Mangum 1982a). In addition, the temperature sensitivity of O₂ binding by hemocyanin is often greater at lower temperatures (Mauro and Mangum, 1982b; Bridges, 1986; Burnett *et al.*, 1988). The hemocyanin of *G. ingens* also seems to have subunits that are quite different electrophoretically from those of decapods (J. E. Reese and C. P. Mangum, pers. comm.), so its properties are of interest for this reason as well.

Although the O₂ binding properties of the hemocyanins of crustaceans inhabiting sometimes hypoxic environments are well studied (Mangum, 1980; Mangum 1983a, b; Morris and Taylor, 1983, 1985; Bridges, 1986), the deep-sea oxygen minima are unique in having stable low oxygen conditions in combination with constant low temperatures. *Gnathophausia ingens*, like most mid-water crustaceans, is denser than seawater (Childress and Nygaard, 1974). Therefore, it must swim continuously and cannot cope with low O₂, or other conditions in its habitat, by becoming quiescent. Because it lives in this habitat continuously, its adaptations must be effective in the context of uninterrupted exposure and activity, clearly a different situation from that facing benthic animals in periodically hypoxic habitats.

The present study of the hemocyanin of *Gnathophausia ingens* was undertaken to determine its functional properties, so that its role in oxygen uptake and transport in this species could be elucidated. In addition, the oxygen binding properties of the hemocyanin of *G. ingens* from off Hawaii were measured to gain insight into the poorer regulation of oxygen consumption found in mysids collected from those waters (Cowles, 1987). The functional properties of hemocyanin from brooding female *G. ingens* (instar 13) were also measured to examine possible ontogenetic changes as individuals move to greater depths and higher values of P_{O₂}.

Materials and Methods

Individuals of *Gnathophausia ingens* were collected during 1985–1988 from San Clemente Basin off southern California, and from the leeward side of the Hawaiian island of Oahu from depths of 400–1200 m. Animals were captured with a modified opening-closing Tucker trawl (3.1 m square mouth), and were brought to the surface in a thermally insulated cod end (Childress *et al.*, 1978), which kept the temperature near 5°C. California animals were kept at 5.5°C on board ship, and were housed (within 3 days of capture) in individual 1-liter containers at 5.5°C at the Santa Barbara laboratory until used in experiments. Except where noted (regional comparisons, Fig. 5), all experiments were conducted with

hemolymph samples from mysids collected off California.

Initial sampling

Hemolymph samples were removed from the ventral sinus and ventral abdominal vessel of *Gnathophausia ingens*. In the measurement of pH, a sample of hemolymph was withdrawn with a syringe and, without air exposure, was immediately injected into a Radiometer glass capillary electrode (Radiometer America G298A) in a water jacketed chamber, in conjunction with a reference electrode (Radiometer K171). Precision buffers were used to calibrate the electrode (Radiometer S1500 and S1510). Samples for oxygen equilibrium curves were dialyzed for immediate use, or frozen for later use.

The concentration of hemocyanin in pooled ($n = 5$) hemolymph samples from *Gnathophausia ingens* (intermediate instar California and Hawaii animals, and brooding females from California) was estimated by measuring the absorbance maximum, near 340 nm, of a 1:100 dilution of hemolymph in 50 mmol/l Tris buffer (pH 8.9) with EDTA (50 mmol/l). Although extinction coefficients have not been determined for mysid hemocyanin, the extinction coefficient for the lobster *Homarus americanus* ($2.69 \times 10^4 \text{ cm}^{-1} \text{ mol}^{-1}$, Nickerson and van Holde, 1971) was used to estimate hemocyanin concentration. The oxygen carrying capacity was estimated from the haemocyanin concentration, assuming a subunit size of 75,000 D.

The concentrations of Na^+ , K^+ , Ca^{2+} , Mg^{2+} , SO_4^{2-} , and Cl^- in the hemolymph of *Gnathophausia ingens* were measured by single column ion chromatography (Sanders and Childress, 1988), using Wescan cation and anion columns.

The effects of temperature on hemolymph pH in vivo

Individual intermediate instars of *Gnathophausia ingens* were placed in separate 1-liter containers of aerated seawater at 2.5°, 5.0°, 5.5°, 7.5°, 9.0°, or 12°C. After an animal had been maintained at its experimental temperature for 4 h, the pH of its hemolymph was measured. These animals had been captured 1–2 weeks prior to their use.

Oxygen equilibrium curves

To determine the effects of pH and temperature on hemocyanin oxygen binding, dialyzed hemolymph samples were used in a thin-layer spectrophotometric system (Childress *et al.*, 1984; Sanders *et al.*, 1988). Because of the effects of freezing on both affinity and cooperativity (described later), fresh (never frozen) hemolymph samples were used for all measurements except those con-

cerned with freezing and with regional differences in hemocyanin properties.

A small sample of dialyzed *Gnathophausia ingens* hemolymph (5–20 μl) was sandwiched between two layers of teflon membrane (0.006 mm) and placed in a gas-tight, water-jacketed chamber. The absorbance spectrum from 300–800 nm of gas-equilibrated samples was monitored with a Tracor Northern diode array spectrophotometer, and the absorbance at 345 nm was recorded at successively higher oxygen partial pressures. Equilibrium was defined by the absence of further changes in the absorbance spectrum; gas partial pressures around the sample were controlled with a Union Carbide mass flow controller. Oxygen concentrations within the gas-tight sample chamber were monitored with a Systech Instruments Zirconium Cell Oxygen Analyzer. The calibration of the oxygen analyzer was checked frequently with 99.99% oxygen gas, air, and 99.999% nitrogen gas. An additional sample of hemolymph was maintained in a gas-tight, water-jacketed tonometer at the same temperature and gas mixture as the O_2 equilibrium curve sample. The pH of this hemolymph sample was measured near the 50% saturation point at the experimental temperature.

Samples used to determine the effects of temperature and pH on hemocyanin oxygen binding were dialyzed against a physiological saline prepared from the inorganic ion concentrations measured in the hemolymph of intermediate instar *Gnathophausia ingens* from off southern California. Samples were dialyzed for 15–18 h in three changes of physiological saline buffered with 0.05 mol/l Tris buffer (1 part hemolymph to 1000 parts of saline). The effects of L-lactate on O_2 binding by hemocyanin were determined by adding 10 μl of 0, 15, or 150 mmol/l L-lactate in physiological saline to dialyzed 100- μl hemolymph samples. The lactate concentrations in these samples were measured after each experiment using a Boehringer L-lactate test kit.

Data analysis

Results are reported as means and standard deviations unless otherwise noted. Analysis of covariance (ANCOVA) was used to test the significance of differences in the intercepts of regressions only when the slopes did not differ significantly. To examine the properties of this hemocyanin at specific pH values, oxygen equilibrium curves were generated from values of P_{50} and n_{50} interpolated (from regression lines describing $\Delta \log P_{50}/\Delta \text{pH}$ and $\Delta n_{50}/\Delta \text{pH}$) for those pH values. Software programs for all data analyses were written by Dr. S. Morris.

Results

Concentrations of hemocyanin and inorganic ions in hemolymph

Hemolymph samples from freshly captured intermediate instars of *Gnathophausia ingens* from off California

Table I

The concentrations (mmol/l) of the major inorganic elements in hemolymph from intermediate instars of *Gnathophausia ingens* from off California and Hawaii, and from brooding *G. ingens* females from off California

Ion	California	Hawaii	Brooding females
Na ⁺	525.2 ± 13.1 (3)	516.3 ± 13.1 (3)	512.8 ± 16.9 (3)
K ⁺	23.3 ± 2.1 (3)	21.8 ± 1.3 (3)	20.9 ± 1.4 (3)
Ca ²⁺	11.6 ± 3.1 (3)	6.6 ± 0.8 (3)	7.2 ± 1.2 (3)
Mg ²⁺	12.5 ± 1.1 (3)	15.4 ± 1.2 (3)	12.8 ± 1.5 (3)
SO ₄ ²⁻	10.1 ± 1.7 (4)	4.5 ± 0.9 (3)	8.7 ± 2.1 (3)
Cl	532.8 ± 16.3 (4)	525.6 ± 14.4 (3)	530.8 ± 21.2 (3)

Values were determined by ion chromatography and are reported as means ± 1 standard deviation, followed by the number of observations in parentheses.

and Hawaii had a hemocyanin concentration of 24 mg/ml (pooled samples of 5 individuals at each site), while the hemocyanin concentration in hemolymph of brooding females from California was lower (16 mg/ml, 5 individuals pooled). The O₂ carrying capacities of the hemocyanin in the hemolymphs were estimated at 0.32 and 0.21 mmol/l, respectively. Hemolymph ion concentrations were typical for a marine crustacean (Mangum, 1983a) and differed little among California, Hawaii, and brooding female *G. ingens* (Table I).

Temperature/pH relationship in vivo

The regression line calculated from *in vivo* pH measurements versus experimental temperature (2.5–12°C) is: pH = 7.95–0.018 (T°C), r² = 0.82, n = 35. The short term *in vivo* pH change due to temperature in the hemolymph of intermediate instar *Gnathophausia ingens* (ΔpH/ΔT = –0.018) was similar to the change due to temperature in the neutral pH of water (ΔpH/ΔT = –0.017, Reeves, 1977). This value is also similar to *in vivo* hemolymph pH changes measured in other crustaceans over physiological temperature ranges (McMahon and Burggren, 1981; Morris *et al.*, 1985, 1988; Morris and Bridges, 1989).

Effects of pH and temperature on oxygen binding by hemocyanin

The effects of pH and temperature (2–15°C) on oxygen binding by hemocyanin in dialyzed, never frozen hemolymph samples from specimens of *Gnathophausia ingens* captured off California are reported in Figure 2. The effect of pH on hemocyanin oxygen affinity was large (Δ log P₅₀/ΔpH = –0.80, 2 to 10°C and –0.81 at 15°C). There was no significant effect of temperature on HCO₂ affinity over the 2–15°C temperature range, as shown by

a comparison of the y-intercepts of the regression lines relating log P₅₀ to pH at the different temperatures (ANCOVA). Temperature also had no significant effect on cooperativity from 5 to 15°C (ANCOVA), but the slope of the relationship between n₅₀ and pH at 2°C was significantly different from those at the higher temperatures. The temperature sensitivity of HCO₂ binding was analyzed by van't Hoff plots (Fig. 3). The data for these plots were obtained by estimating P₅₀ values at three constant values of pH from the regression analyses (Δ log P₅₀/ΔpH) of data reported in Figure 2. The low ΔH values at constant pH (ΔH = –6.7 kJ/mol, pH 7.7, 2–10°C) emphasize the lack of temperature sensitivity in this species in the physiological temperature range (Fig. 3).

Effects of L-lactate on oxygen binding by hemocyanin

The effects of L-lactate at 5°C on HCO₂ binding in dialyzed hemolymph (never frozen) of *Gnathophausia ingens* from California are shown in Figure 4. The slopes of the log P₅₀ versus pH regressions for 0.09 and 14.32 mmol/l L-lactate were not significantly different, but the elevations were (P < 0.005, ANCOVA). Thus lactate significantly increases the affinity of this hemocyanin for O₂ (Δ log P₅₀/Δ log [lactate] = –0.17, 5.0°C, pH 7.9). The corresponding regression line at 1.23 mmol/l L-lactate fell in between the other two, but its slope was significantly different from those of the other two (P < 0.025). Analysis of covariance showed that cooperativity of oxy-

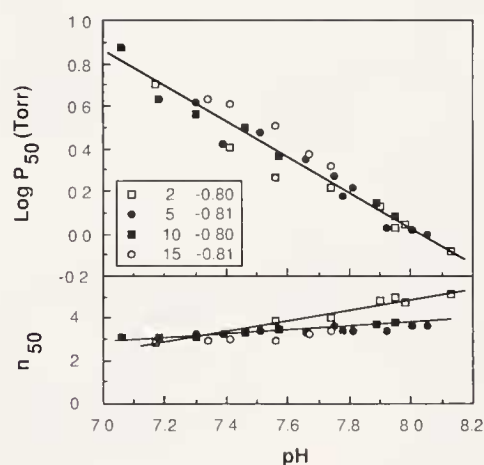


Figure 2. The effects of pH and temperature on HCO₂ binding in dialyzed (never frozen) hemolymph samples from intermediate instar *Gnathophausia ingens* from California. Regression equations for oxygen affinity of hemocyanin: 2°C, Log P₅₀ = 6.04 – 0.80 pH, r² = 0.96; 5°C, Log P₅₀ = 6.50 – 0.81 pH, r² = 0.94; 10°C, Log P₅₀ = 6.47 – 0.80 pH, r² = 0.98; 15°C, Log P₅₀ = 6.59 – 0.81 pH, r² = 0.98. Regressions for cooperativity (lines for 2°C and 5°C): 2°C, n₅₀ = –14.83 + 2.47 pH, r² = 0.96; 5°C, n₅₀ = –0.89 + 0.56 pH, r² = 0.70; 10°C, n₅₀ = –3.22 + 0.88 pH, r² = 0.98; 15°C, n₅₀ = –5.50 + 1.14 pH, r² = 0.76.

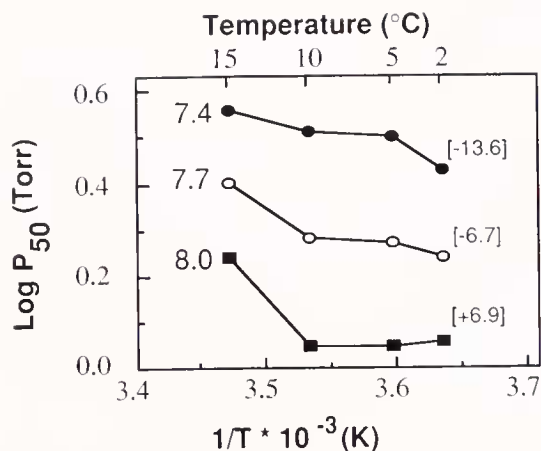


Figure 3. The effect of temperature (2–15°C) on HcO₂ binding of *Gnathophausia ingens* at three constant values of pH (7.4, 7.7, 8.0). Hemolymph samples were taken from intermediate instars of *G. ingens* from California. Numbers in brackets are ΔH values calculated from Log P₅₀/pH data (Fig. 1) at the indicated pH over the 2–10°C temperature range. Points plotted are interpolations from the data in Figure 2.

gen binding by hemocyanin was significantly increased ($P < 0.05$) in the presence of L-lactate (Fig. 4).

Effects of freezing and regional differences on oxygen binding by hemocyanin

When compared with hemolymph samples that had not been frozen, samples from California *Gnathophausia*

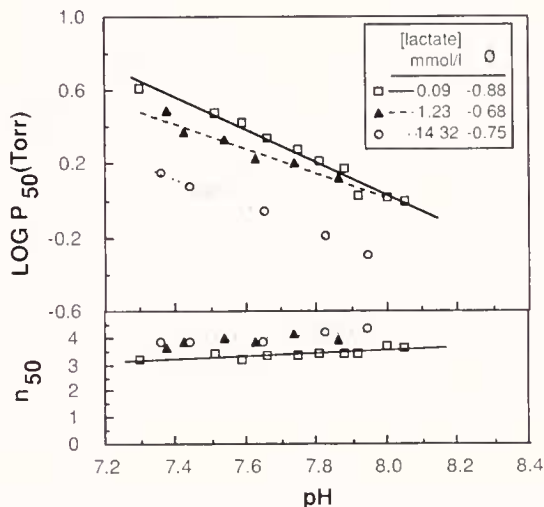


Figure 4. The effects of L-lactate on HcO₂ binding at 5°C of intermediate instars of *Gnathophausia ingens* from California. Regression equations for HcO₂ affinity: 0.09 mmol l⁻¹ L-lactate, Log P₅₀ = 6.50 – 0.81 pH, $r^2 = 0.94$; 1.23 L-lactate, Log P₅₀ = 3.42 – 0.62 pH, $r^2 = 0.89$; 14.32 L-lactate, Log P₅₀ = 5.70 – 0.75 pH, $r^2 = 0.99$. Regression equations for cooperativity (lines plotted for 0.09 and 14.32 mmol/l L-lactate): 0.09 mmol l⁻¹ L-lactate, $n_{50} = -0.83 + 0.55$ pH, $r^2 = 0.82$; 1.23 L-lactate, $n_{50} = -2.15 + 0.81$ pH, $r^2 = 0.79$; 14.32 L-lactate, $n_{50} = -3.88 + 0.81$ pH, $r^2 = 0.99$.

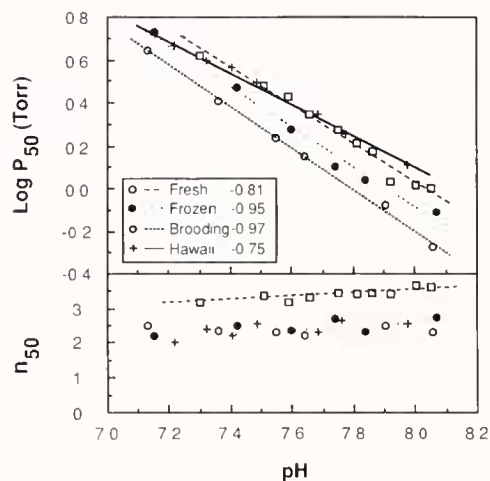


Figure 5. HcO₂ affinity and cooperativity at 5°C in fresh and frozen hemolymph from intermediate instar and brooding female *Gnathophausia ingens* from California, and from intermediate instar Hawaiian *G. ingens*. Regressions for each affinity line: California fresh (fresh), Log P₅₀ = 6.50 – 0.81 pH, $r^2 = 0.94$; California frozen (frozen), Log P₅₀ = 7.51 – 0.95 pH, $r^2 = 0.99$; California brooding female frozen (brood), Log P₅₀ = 7.53 – 0.97 pH, $r^2 = 0.99$; Hawaii frozen (Hawaii), Log P₅₀ = 6.06 – 0.75 pH, $r^2 = .99$. Regressions for cooperativity (lines for California fresh and California frozen): California fresh (fresh), $n_{50} = -0.83 + 0.55$ pH, $r^2 = 0.82$; California frozen (frozen), $n_{50} = -0.81 + 0.43$ pH, $r^2 = 0.99$; California brooding female frozen (brooding), $n_{50} = -0.30 - 0.07$ pH, $r^2 = 0.79$; Hawaii frozen (Hawaii), $n_{50} = -1.93 + 0.57$ pH, $r^2 = .99$.

sia ingens that had been stored at –80°C for 18–24 months contained hemocyanin with significantly increased O₂ affinity (5°C, $P < 0.05$, ANCOVA, Fig. 5) and significantly decreased cooperativity ($P < 0.05$, ANCOVA). Therefore, we used hemolymph that had never been frozen for all of the other studies on the HcO₂ binding properties of intermediate instar individuals from California. However, because of the availability of frozen but not fresh material, ontogenetic and regional comparisons were made on frozen samples.

When the HcO₂ binding of frozen, dialyzed hemolymph samples from California brooding females was compared with that of intermediate instars of *G. ingens*, the hemocyanin from the brooding females, had a significantly higher affinity at 5°C, but was not significantly different in cooperativity (ANCOVA, Fig. 5). The hemocyanin in frozen, dialyzed hemolymph samples from Hawaiian intermediate instar *G. ingens* had significantly lower O₂ affinity at 5°C than did that from the California intermediate instars: there was no significant difference, however, in the cooperativity of HcO₂ binding (ANCOVA, Fig. 5). Although the effects of freezing may compromise these results, if freezing had affected all samples equally, then these results would still suggest that ontogenetic changes occur in the hemocyanins of *G. ingens* and that the Hawaiian individuals of this species

living at higher O₂ partial pressures have hemocyanin with a lower O₂ affinity than do California individuals. Studies on fresh material will be necessary to confirm these suggestions.

Discussion

Adaptations to the oxygen minimum layer

Gnathophausia ingens, like most pelagic crustaceans occupying deep-sea oxygen minima, relies primarily on aerobic metabolism, supported by unusually well-developed abilities to remove O₂ from water, to exploit these vast, stable, low O₂ environments (Childress, 1975). Although this species has very little capacity to live without O₂ (it survives for less than 30 min under anoxic conditions), it can regulate its oxygen uptake (P_c as low as 3 torr O₂ at minimal activity) at least to its lowest environmental P_{O₂} (6 torr) when it is routinely active (Childress, 1971). *G. ingens* can regulate its O₂ consumption to such low P_{O₂} values because it maintains a very high ventilatory flow (up to 8 body volumes/min) and a high rate of removal of O₂ from the ventilatory stream (50–80%), even at the lowest P_{O₂} in its environment (Childress, 1971).

The high removal is made possible by great development of the gills and circulatory system in this mysid. The gill surface area is quite large (9–14 cm²/g wet wt.) for a crustacean, and the oxygen diffusion distance across the branchial epithelium (about 2 μm) is smaller than that measured in most other crustaceans (Belman and Childress, 1976; Taylor, 1982; McMahon and Wilkens, 1983). Therefore, the total oxygen diffusing capacity of the gills of *G. ingens* (as calculated by Belman and Childress, 1976), is much greater than that estimated for other crustaceans, allowing a rate of diffusion of O₂ across the gills that is sufficient even with the very limited O₂ available in its environment. Indeed, a P_{O₂} difference of only about 3–4 torr across the gills was calculated to be sufficient to allow the diffusion of enough O₂ to support routine metabolism. The heart and arterial channels of *G. ingens* are quite large for a crustacean of its size, and the heart also can generate a relatively high systolic pressure for a crustacean of its small size (Belman and Childress, 1976; McMahon and Wilkens, 1983). These properties enable *G. ingens* to generate a high rate of hemolymph flow (55–225 ml/kg/min) as compared to other crustaceans, providing rapid turnover of oxygen depleted venous hemolymph (Belman and Childress, 1976; McMahon and Wilkens, 1983).

However, even with these circulatory adaptations, Belman and Childress (1976) estimated that, without a functional O₂ binding protein in the hemolymph, an unreasonably high value for blood flow (more than 4 times the known maximum values) would be necessary to supply

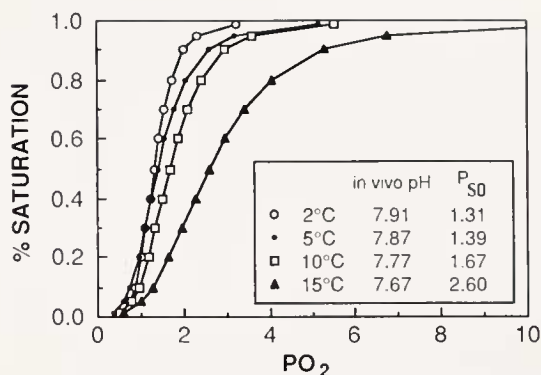


Figure 6. *Gnathophausia ingens* HcO₂ equilibrium curves generated from data in Figure 2, using predicted values of *in vivo* pH [see regression: $\Delta\text{pH}/\Delta T$ (°C) in results section] at particular temperatures. The environmentally appropriate temperature for the intermediate instars of this species is 5°C.

the oxygen needed to sustain aerobic respiration in *G. ingens* at 6 torr O₂. To maintain the O₂ consumption rate reported (Childress, 1968, 1971), the mysid would need between 9–36 times greater hemolymph oxygen carrying capacity (0.10–0.78 mmol/l) than could be explained by the maximum dissolved oxygen present at 6 torr (0.011 mmol/l), assuming 100% use of bound O₂ (Belman and Childress, 1976). The hemocyanin oxygen carrying capacity, estimated for California *G. ingens* from measurements of hemocyanin concentration, is 0.32 mmol/l, in good agreement with the estimate of Belman and Childress (1976).

In order to be an effective transporter of O₂, a respiratory protein must be able to load O₂ to near saturation when in the gills, and to unload a large fraction of this O₂ at the tissues. The O₂ binding properties of *G. ingens* hemocyanin can be evaluated for the extent to which they appear to be adaptive in this context. The very high affinity for O₂ should enable the hemocyanin of *Gnathophausia ingens* to bind oxygen from the low P_{O₂} water of the oxygen minimum layer. Because this hemocyanin is 95% saturated at 3 torr O₂ and physiological temperatures (5°C, pH 7.87, Fig. 6), its affinity is high enough to maintain the necessary O₂ gradient of 3 to 4 torr across the gills to support routine metabolism (Belman and Childress, 1976). The high cooperativity at low temperature of this hemocyanin enables it to release most of its bound O₂ with a drop in P_{O₂} of only 2 torr (5°C, pH 7.87, Fig. 6). The large Bohr shift of this hemocyanin would be expected to make this unloading even more effective.

The increase in O₂ affinity by lactate might well be adaptive in this species, as it is in *Callinectes sapidus*, compensating for decreases in affinity resulting from lowered pH during periods of maximal activity, which would have to be partially anaerobically fueled at 6 torr O₂ (Childress, 1971; Booth *et al.* 1982). The concentra-

tion of *G. ingens* hemocyanin is among the lowest ones measured (Mangum, 1983a). However, at the very low P_{O_2} values at which this mysid lives, this O_2 carrying capacity is far greater than the dissolved O_2 . The O_2 affinity of *G. ingens* hemocyanin, being temperature insensitive, is unaffected by the small temperature changes within the species' vertical range—a possible adaptation. Given the narrow range of P_{O_2} over which this hemocyanin functions, even small temperature-caused shifts in affinity could have detrimental effects on O_2 transport if the hemocyanin were not temperature insensitive. A low thermal sensitivity of HcO_2 affinity appears to be usual for the hemocyanins of non-hydrothermal vent deep-sea decapods, as well as mysids (Arp and Childress, 1985; Sanders, 1989). Thus, the O_2 binding properties of its hemocyanin appear to be highly adaptive in supporting the uptake and transport of O_2 at the normal low P_{O_2} values in the environment of *G. ingens*.

Patterns of functional properties of hemocyanins

The literature on crustacean hemocyanins deals almost exclusively with shallow water decapods, particularly brachyurans. The oxygen binding properties of hemocyanin have been considered to be conservative in expression (Mangum, 1980), yet plastic in their inherent ability to adapt to environmental conditions. Temperature is an environmental variable with direct and indirect effects on HcO_2 binding, and several generalizations, concerning the effects of temperature on hemocyanins—and based almost entirely on data for shallow living species—have been stated. One such generalization is that the oxygen affinity of hemocyanins is lower in species from “low” temperature environments to offset the usual effect of lower temperature, an increase in the affinity of hemocyanin (Redmond, 1968, Mangum, 1982, Mauro and Mangum, 1982a). The oxygen affinities of hemocyanins from deep-sea pelagic crustaceans do not fit this generalization, however, and they would not be functional in the low oxygen environment found in midwater zones if this were the case (Sanders, 1989). Instead, the quite low P_{O_2} appears to be the selective factor most strongly affecting the functional properties of hemocyanins from O_2 minimum layers, resulting in high HcO_2 affinity and reduced temperature sensitivity. This does support a second generalization concerning temperature and HcO_2 affinity, which suggests an inverse relationship between the Bohr shift (which is large for *Gnathophausia ingens*) and temperature sensitivity (Burnett *et al.*, 1988). Therefore, crustacean hemocyanins seem to have a considerable degree of adaptive plasticity. In those cases that do not follow the expected patterns—*e.g.*, the absence of temperature sensitivity of *G. ingens* Hc, the reverse temperature sensitivity found in *Palae-*

mon elegans Hc (Morris *et al.* 1985), and hydrothermal vent crab Hc (Sanders *et al.*, 1988; Sanders, 1989)—the hemocyanins appear to be adapted for the particular habitat and habits of the organisms.

Ontogenetic differences

The life history characteristics of *Gnathophausia ingens* are discussed in detail by Childress and Price (1978). Briefly, females of *G. ingens* reproduce only once, and brood the eggs and young in a marsupium for about 530 days. There are 13 distinct instars in this species, and the newly released juveniles (instars 3 and 4) are found at 175–300 m depth (40–100 torr O_2). Intermediate instars (numbers 5 to 10) are found at 650–750 m by day (6–10 torr O_2 off California), and disperse between 400–800 m (6–22 torr O_2) by night. Brooding females (carrying the first 2 instars) live at depths between 800 and 1400 m (10–20 torr O_2). Given the large changes in depth, and therefore temperature and P_{O_2} , during the life of an individual *G. ingens*, one might expect ontogenetic changes in its hemocyanin. The brooding females, in particular, have much lower metabolic rates and live at lower temperatures and slightly higher P_{O_2} values. The hemocyanin concentration of brooding females is reduced relative to that of intermediate instars living at shallower depths and, based on frozen material, the oxygen affinity of the hemocyanin from the brooding female appears to be significantly higher (Fig. 4). The lower O_2 carrying capacity is consistent with the lower metabolic rate and activity of this instar (Childress, 1975).

Regional differences

Gnathophausia ingens is also found off the Hawaiian islands. The depth distribution of the population of *G. ingens* sampled off Oahu was essentially identical to that of *G. ingens* collected in the basins off southern California. Thus, the animals in Hawaiian waters are exposed to much higher P_{O_2} (approximately 20 torr at day-time depths, and greater than 100 torr at night) than those off southern California. Hawaiian individuals of *G. ingens* cannot regulate O_2 consumption as well as can individuals from California ($P_c = 10$ –30 torr, depending upon oxygen consumption rate, for Hawaiian *G. ingens* individuals, Cowles, 1987). Although there is no significant difference in the oxygen consumption rates, the relationship between oxygen consumption and P_c in the two populations is significantly different. A cursory anatomical comparison of the Hawaiian and Californian *G. ingens* individuals revealed no obvious differences in the circulatory system or gills (J. Childress, unpub. obs.). The hemocyanin concentrations in the hemolymphs of *G. ingens* individuals from these two regions are comparable, but the differences in the functional properties of

the hemocyanins (*e.g.*, the apparently lower O₂ affinity of hemocyanin from Hawaiian animals based on frozen material) may explain, at least in part, the observed differences in the regulation of oxygen consumption. Thus, different populations of the same species, *G. ingens*, in different oceanic regions may have adaptive differences in the functional properties of their hemocyanins. Adaptive regional differences in the O₂ affinities of the hemocyanin of *Callinectes sapidus* result from changes in subunit composition due to acclimation (Mangum and Rainer, 1988).

In summary, the high oxygen affinity of the hemocyanin of the mysid *Gnathophausia ingens* appears to be essential for these pelagic, permanent residents of deep-sea oxygen minimum layers. The high affinity and cooperativity of oxygen binding by their hemocyanin, and the previously reported circulatory and ventilatory adaptations, apparently enable the hemocyanin to be fully saturated with oxygen at very low external values of PO₂, and to release a large percentage of the bound oxygen at the tissues with a very small change in PO₂. Offloading is potentially further facilitated by the presence of a large Bohr effect. This hemocyanin clearly shows a different combination of properties than is found in crustaceans from other habitats. However, these HCO₂ binding properties, are highly adaptive for this species in the O₂ minimum layer habitat. While the oxygen binding properties of hemocyanins from shallow-living species are conservative in expression, the inherent adaptive plasticity of crustacean hemocyanins may be considerably greater than has previously been appreciated.

Acknowledgments

This research was supported by N.S.F. grant OCE 8500237 to J.J.C. and a N.S.F. Predoctoral Fellowship to N.K.S. Special thanks to the captains and crews of the research vessels New Horizon and Pt. Sur for assistance with animal collection. We thank A. Alldredge, A. Kuris, S. Morris, and B. Robison for critical review of earlier versions of this manuscript.

Literature Cited

- Arp, A. J., and J. J. Childress. 1985. Oxygen binding properties of the blood of the deep-sea shrimp, *Glyphocrangon vicaria*. *Physiol. Zool.* **58**: 38–45.
- Banse, K. 1964. On the vertical distribution of zooplankton in the sea. Pp. 53–1225 in *Progress in Oceanography, Volume II*, M. Sears, ed. Pergamon Press, Oxford.
- Belman, B. W., and J. J. Childress. 1976. Circulatory adaptations to the oxygen minimum layer in the bathypelagic mysid *Gnathophausia ingens*. *Biol. Bull.* **150**: 15–37.
- Bridges, C. R. 1986. A comparative study of the respiratory properties and physiological function of haemocyanin in two burrowing and two non-burrowing crustaceans. *Comp. Biochem. Physiol.* **83A**: 261–270.
- Booth, C. E., B. R. McMahon, and A. W. Pinder. 1982. Oxygen uptake and the potentiating effects of increased hemolymph lactate on oxygen transport during exercise in the blue crab, *Callinectes sapidus*. *J. Comp. Physiol.* **148**: 111–121.
- Burnett, L. E., D. A. Scholnick, and C. P. Mangum. 1988. Temperature sensitivity of molluscan and arthropod hemocyanins. *Biol. Bull.* **174**: 153–162.
- Childress, J. J. 1968. Oxygen minimum layer: vertical distribution and respiration of the mysid *Gnathophausia ingens*. *Science* **160**: 1242–1243.
- Childress, J. J. 1971. Respiratory adaptations to the oxygen minimum layer in the bathypelagic mysid *Gnathophausia ingens*. *Biol. Bull.* **141**: 109–121.
- Childress, J. J. 1975. The respiratory rates of midwater crustaceans as a function of depth of occurrence and relation to the oxygen minimum layer off Southern California. *Comp. Biochem. Physiol.* **50**: 787–799.
- Childress, J. J., and M. Nygaard. 1974. The chemical composition and buoyancy of midwater crustaceans as a function of depth of occurrence off southern California. *Mar. Biol.* **27**: 225–238.
- Childress, J. J., and M. H. Price. 1978. Growth rate of the bathypelagic crustacean *Gnathophausia ingens* (Mysidacea: Lophogastridae). I. Dimensional growth and population structure. *Mar. Biol.* **50**: 47–62.
- Childress, J. J. and M. H. Price. 1983. Growth rate of the bathypelagic crustacean *Gnathophausia ingens* (Mysidacea: Lophogastridae). II. Accumulation of material and energy. *Mar. Biol.* **76**: 165–177.
- Childress, J. J., A. J. Arp, and C. R. Fisher. 1984. Metabolic and blood characteristics of the hydrothermal vent tube-worm *Riftia pachyptila*. *Mar. Biol.* **83**: 109–124.
- Childress, J. J., A. T. Barnes, L. B. Quetin, and B. H. Robison. 1978. Thermally protecting cod ends for the recovery of living deep-sea animals. *Deep-Sea Res.* **25**: 419–422.
- Cowles, D. L. 1987. Factors affecting the aerobic metabolism of midwater crustaceans. Ph.D. dissertation, University of California, Santa Barbara. 228 pp.
- Freel, R. W. 1978. Oxygen affinity of the hemolymph of the mesopelagic mysidacean *Gnathophausia ingens*. *J. Exp. Zool.* **204**: 267–273.
- McMahon, B. R., and W. W. Burggren. 1981. Acid-base balance following temperature acclimation in land crabs. *J. Exp. Zool.* **218**: 45–52.
- McMahon, B. R., and J. L. Wilkens. 1983. Ventilation, perfusion, and oxygen uptake. Pp. 290–372 in *The Biology of the Crustacea, Vol. 5: Internal Anatomy and Physiological Regulation*, L. H. Mantel, ed. Academic Press, New York.
- Mangum, C. P. 1980. Respiratory function of the hemocyanins. *Am. Zool.* **20**: 19–38.
- Mangum, C. P. 1982. On the relationship between P₅₀ and the mode of gas exchange in tropical crustaceans. *Pac. Sci.* **36**: 403–410.
- Mangum, C. P. 1983a. Oxygen transport in the blood. Pp. 373–429 in *The Biology of the Crustacea, Vol. 5: Internal Anatomy and Physiological Regulation*, L. H. Mantel, ed. Academic Press, New York.
- Mangum, C. P. 1983b. The effect of hypoxia on hemocyanin-oxygen binding in the horseshoe crab *Limulus polyphemus*. *Molec. Physiol.* **3**: 217–224.
- Mangum, C. P., and J. S. Rainer. 1988. The relationship between subunit composition and O₂ binding of blue crab hemocyanin. *Biol. Bull.* **174**: 77–82.
- Mauro, N. A., and C. P. Mangum. 1982a. The role of blood in the temperature dependence of oxidative metabolism in decapod crustaceans. I. Intraspecific responses to seasonal differences in temperature. *J. Exp. Zool.* **219**: 179–188.

- Mauro, N. A., and C. P. Mangum. 1982b. The role of blood in the temperature dependence of oxidative metabolism in decapod crustaceans. II. Interspecific adaptations to latitudinal changes. *J. Exp. Zool.* **219**: 189–195.
- Morris, S. M., and A. C. Taylor. 1983. Diurnal and seasonal variation in physico-chemical conditions within intertidal rock pools. *Estuar. Coast Shelf Sci.* **17**: 151–167.
- Morris, S. M., and A. C. Taylor. 1985. The respiratory response of the intertidal prawn *Palaemon elegans* (Rathke) to hypoxia and hyperoxia. *Comp. Biochem. Physiol.* **81A**: 633–639.
- Morris, S. M., A. C. Taylor, C. R. Bridges, and M. K. Grieshaber. 1985. Respiratory properties of the haemolymph of the intertidal prawn *Palaemon elegans* (Rathke). *J. Exp. Zool.* **233**: 175–186.
- Morris, S. M., P. Greenaway, and B. R. McMahon. 1988. Oxygen and carbon dioxide transport by the haemocyanin of an amphibious crab, *Holthuisana transversa*. *J. Comp. Physiol.* **157B**: 873–882.
- Nickerson, K. W., and K. E. van Holde. 1971. A comparison of molluscan and arthropod hemocyanin. I. Circular dichroism and absorption spectra. *Comp. Biochem. Physiol.* **39B**: 855–872.
- Redmond, J. R. 1968. The respiratory function of hemocyanin. Pp. 5–23 in *Biochemistry and Physiology of Hemocyanins*, G. Ghirelli, ed. Academic Press, New York.
- Reeves, R. B. 1977. The interaction of body temperature and acid-base balance in ectothermic vertebrates. *Ann. Rev. Physiol.* **39**: 559–586.
- Sanders, N. K. 1989. Functional properties of hemocyanins from deep-sea crustaceans. *Ph.D. thesis, University of California, Santa Barbara*. 209 pp.
- Sanders, N. K., and J. J. Childress. 1988. Ion replacement as a buoyancy mechanism in a pelagic deep-sea crustacean. *J. Exp. Biol.* **138**: 333–343.
- Sanders, N. K., A. J. Arp, and J. J. Childress. 1988. Oxygen binding characteristics of the hemocyanins of two deep-sea hydrothermal vent crustaceans. *Respir. Physiol.* **71**: 57–68.
- Schmidt, J. 1925. On the contents of oxygen in the ocean on both sides of Panama. *Science* **61**: 592–593.
- Sewell, R. B. S., and L. Fage. 1948. Minimum oxygen layer in the ocean. *Nature* **162**: 949–951.
- Taylor, E. W. 1982. Control and co-ordination of ventilation and circulation in crustaceans: responses to hypoxia and exercise. *J. Exp. Biol.* **100**: 289–319.